5. DOSE-RESPONSE CHARACTERIZATION

Previous sections of this integrated summary have focused on characterizing the hazards of and exposure to dioxin-like compounds. In order to bring these issues together and provide an adequate characterization of risk, the relationships of exposure to dose and, ultimately, to response must be evaluated. Key questions to be asked include: (1) What can be said about the shape of the dose-response function in the observable range and what does this imply about dose-response in the range of environmental exposures? (2) What is a reasonable limit (critical dose or point of departure) at the lower end of the observable range and what risk is associated with this exposure? In addition, one can address the issue of extrapolation beyond the range of the data in light of the answers to the above questions. Although extrapolation of risks beyond the range of observation in animals and/or humans is an inherently uncertain enterprise, it is recognized as an essential component of the risk assessment process (NAS/NRC, 1983). The level of uncertainty is dependent on the nature (amount and scope) of the available data and on the validity of the models that have been used to characterize dose-response. These form the

bases for scientific inference regarding individual or population risk beyond the range of current observation ((NAS/NRC, 1983, 1994)

In Part II, Chapter 8, the body of literature concerning dose-response relationships of TCDD is presented. This chapter addresses the important concept of selecting an appropriate metric for cross-species scaling of dose and presents the results of empirical modeling for many of the available data sets on TCDD exposures in humans and in animals. Although not all human observations or animal experiments are amenable to dose-response modeling, more than 200 data sets were evaluated for shape, leading to an effective dose (ED) value expressed as a percent response being presented for the endpoint being evaluated (e.g., ED_{01} equals an effective dose for a 1% response). The analysis of dose-response relationships for TCDD, considered within the context of toxicity equivalence, mechanism of action, and background human exposures, helps to elucidate the common ground and the boundaries of the science and science policy components inherent in this risk characterization for the broader family of dioxin-like compounds. For instance, the dose-response relationships provide a basis to infer a point of departure for extrapolation for cancer and noncancer risk for a complex mixture of dioxin-like congeners given the assumption of toxicity equivalence as discussed in Part II, Chapter 9. Similarly, these relationships provide insight into the shape of the dose-response at the point of departure, which can help inform choices for extrapolation models for both TCDD and total TEQ.

In evaluating the dose-response relationships for TCDD as a basis for assessing this family of compounds, both empirical dose-response modeling approaches and mode-of-action-based approaches have been developed and applied (see Part II, Chapter 8; Portier et al., 1996). Empirical models have advantages and disadvantages relative to more ambitious mechanism-based models. Empirical models provide a simple mathematical model that adequately describes the pattern of response for a particular data set; they can also provide the means for hypothesis testing and interpolation between data points. In addition, they can provide qualitative insights into underlying mechanisms. However, the major disadvantage of empirical models is their inability to quantitatively link data sets in a mechanistically meaningful manner. On the other hand, mechanism-based modeling can be a powerful tool for understanding and combining information on complex biological systems. Use of a truly mechanism-based approach can, in theory, enable more reliable and scientifically sound extrapolations to lower doses and between species. However, any scientific uncertainty about the mechanisms that the models describe is inevitably reflected in uncertainty about the predictions of the models.

Physiologically based pharmacokinetic (PBPK) models have been validated in the observable response range for numerous compounds in both animals and humans. The development of PBPK models for disposition of TCDD in animals has proceeded through multiple levels of refinement, with newer models showing increasing levels of complexity by incorporating

data for disposition of TCDD, its molecular actions with the AhR and other proteins, as well as numerous physiological parameters (Part II, Chapter 1). These have provided insights into key determinants of TCDD disposition in treated animals. The most complete PBPK models give similar predictions about TCDD tissue dose metrics. The PBPK models have been extended to generate predictions for early biochemical consequences of tissue dosimetry of TCDD, such as induction of CYP1A1. Nevertheless, extension of these models to more complex responses is more uncertain at this time. Differences in interpretation of the mechanism of action lead to varying estimates of dose-dependent behavior for similar responses. The shape of the dose-response curves governing extrapolation to low doses are determined by these hypotheses and assumptions.

At this time, the knowledge of the mechanism of action of dioxin, receptor theory, and the available dose-response data do not firmly establish a scientific basis for replacing a linear procedure for estimating cancer potency. Consideration of this same information indicates that the use of different procedures to estimate the risk of exposure for cancer and noncancer endpoints may not be appropriate. Both the cancer and noncancer effects of dioxin appear to result from qualitatively similar modes of action. Initial steps in the process of toxicity are the same and many early events appear to be shared. Thus, the inherent potential for low dose significance of either type of effect (cancer or noncancer) should be considered equal and evaluated accordingly. In the observable range around 1% excess response, the quantitative differences are relatively small. Below this response, the different mechanisms can diverge rapidly. The use of predicted biochemical responses as dose metrics for toxic responses is considered a potentially useful application of these models. However, greater understanding of the linkages between these biochemical effects and toxic responses is needed to reduce the potentially large uncertainty associated with these predictions.

5.1. DOSE METRIC(s)

One of the most difficult issues in risk assessment is the determination of the dose metric to use for animal-to-human extrapolations. To provide significant insight into differences in sensitivity among species, an appropriate animal-to-human extrapolation of tissue dose is required. The most appropriate dose metric should reflect both the magnitude and frequency of exposure, and should be clearly related to the toxic endpoint of concern by a well-defined mechanism. This is, however, often difficult because human exposures with observable responses may be very different from highly controlled exposures in animal experiments. In addition, comparable exposures may be followed by very different pharmacokinetics (absorption, distribution, metabolism and/or elimination) in animals and humans. Finally, the sequelae of exposure in the form of a variety of responses related to age, organ, and species sensitivity

complicate the choice of a common dose metric. Despite these complexities, relatively simple default approaches, including body surface or body weight scaling of daily exposures, have often been recommended (U.S. EPA, 1992, 1996).

Given the data available on dioxin and related compounds, dose can be expressed in a multitude of metrics (DeVito et al., 1995) such as daily intake (ng/kg/d), current body burden (ng/kg), average body burden over a given period of time, plasma concentration, etc. Examples of other dose metrics of relevance for TCDD and related compounds can be found in the literature including concentration of occupied AhR (Jusko, 1995), induced CYP1A2 (Andersen et al., 1997; Kohn et al., 1993) and reduced epidermal growth factor receptor (EGFR) (Portier and Kohn, 1996). Considering the variety of endpoints seen with TCDD and expected with other dioxin-like chemicals in different species, it is unlikely that a single dose metric will be adequate for interspecies extrapolation for all of these endpoints. The issue of an appropriate dose metric for developmental effects considering the potential for a narrow time window of sensitivity, for instance, has been discussed in a number of places in this document. Furthermore, the use of different dose metrics with respect to the same endpoint may lead to widely diverse conclusions. This latter point is discussed in more detail in Part II, Chapter 8. Nevertheless, it is possible to express dose in a form that allows for comparison of responses for selected endpoints and species. This can be done by choosing a given exposure and comparing responses (e.g., URL) or choosing a particular response level and comparing the associated exposures (e.g., ED).

As discussed above, dose can be expressed in a number of ways. For TCDD and other dioxin-like compounds, attention has focused on the consideration of dose expressed as daily intake (ng/kg/day), body burden (ng/kg), or AUC (DeVito et al, 1995; Aylward et al, 1996). The concept of physiological time (lifetime of an animal) complicates the extrapolation, as the appropriate scaling factor is uncertain for toxic endpoints. Because body burden incorporates differences between species in TCDD half-life (these differences are large between rodent species and humans [Table 8.2], this dose metric appears to be the most practical for this class of compounds (DeVito et al, 1995). Average lifetime body burden is best suited for steady-state conditions, with difficulties arising when this dose metric is applied to evaluation of acute exposures, such as those occurring in the 1976 accidental exposure of some people living in Seveso, Italy (Bertazzi and di Domenico, 1994). In cases such as this, increased body burden associated with the acute exposure event is expected to decline (half-life for TCDD is approximately 7 years) until it begins to approach a steady-state level associated with the much smaller daily background intake. However, this issue of acute exposure is not a major factor in the current analyses. In general, daily excursions in human exposure are relatively small and have minor impact on average body burden. Instead, PBPK models suggest that human body burdens increase over time and begin to approach steady-state after approximately 25 years with typical

background doses. Occupational exposures represent the middle ground where daily excursions during the working years can significantly exceed daily background intakes for a number of years, resulting in elevated body burdens. This is illustrated in Table 5-1. Estimation of the range and mean or median of "attained" body burden in accidentally or occupationally exposed cohorts is presented and compared with body burdens based on background exposures. These data are presented graphically in Figure 5-1.

Table 5-1 and Figure 5-1 summarize literature on levels of dioxin TEQs in the background human population and in commonly cited epidemiological cohorts. Table 5-1 collates data on tissue lipid levels (ppt lipid adjusted) in populations, principally from serum, tabulating either current levels for the background population or back calculated levels for the exposed cohorts. Figure 5-1 graphs the estimated range and central tendency of the total TEQ_{DFP} body burden (ng/kg whole body), combining the range of measured 2,3,7,8-TCDD values with the estimate of the background non-2,3,7,8-TCDD TEQ level from the U.S. population in the late 1980s/early 1990s. TEQ levels are calculated for PCDD, PCDF, and PCBs, based on TEQ_{DFP}-WHO_{98} values, and assume a constant 25% body fat ratio when converting from serum lipid ppt to ng/kg body burden. Total TEQ values for the Hamburg cohort women were calculated by the authors, and for this cohort the TCDD graph includes non-TCDD TEQ. Seveso values reported by Needham et al. (1999) are based on stored serum samples from subjects undergoing medical examinations contemporaneous with the exposure, and were not back-calculated.

For the background U.S. populations (CDC; USA ~1990s), the bars represent the range of total TEQ measured in the population. The lower shaded portion represents the variability from non-2,3,7,8-TCDD derived TEQs, the upper shaded portion the variability in the 2,3,7,8-TCDD. Note, that the respective bar sizes do not represent the total non-2,3,7,8-TCDD TEQ or 2,3,7,8-TCDD contributions, because a portion of each of these contributions is contained within the region between the x-axis and bottom of the bar, namely the minimum estimated body burden. For each of the back-calculated epidemiological cohort exposures, the bar was estimated based on the combination of two distributions: the 2,3,7,8-TCDD levels measured in the respective cohort plus the estimated range of background non-2,3,7,8-TCDD derived TEQs from the U.S. population. The lower estimate is the combination of the lower 2,3,7,8-TCDD and lower non-2,3,7,8-TCDD TEQ contributions; the shading junction represents the variability in background U.S. population non-2,3,7,8-TCDD levels that have been added to this bar; the mean/median/geometric mean indicators represent the addition of the measured 2,3,7,8-TCDD central estimate with the mean background US population non-2,3,7,8-TCDD TEQ level (~47.6 ppt lipid, 11.9 ng/kg body burden at 25% body fat); and the upper limit is the combination of the upper 2,3,7,8-TCDD and upper non-2,3,7,8-TCDD TEQs.

As discussed earlier, using background of total body burden (TEQ_{DFP}-WHO_{98}) as a point of comparison, these often- termed "highly exposed" populations have maximum body burdens that are relatively close to general population backgrounds at the time. When compared to background body burdens of the late 1980s, many of the median values and some of the mean values fall within a range of one order of magnitude (factor of 10) and all fall within a range of two orders of magnitude (factor of 100). General population backgrounds at the time are likely to have been higher. As these are attained body burdens, measured at the time of the Seveso accident or back-calculated to the time of last known elevated exposure, being compared to backgound, average lifetime body burdens in these cohorts will be even closer to lifetime average background levels. This will be important if, as demonstrated for some chronic effects in animals and as assumed when relying on average body burden as a dose metric, cancer and other noncancer effects are a consequence of average tissue levels over a lifetime. Body burdens begin to decline slowly soon after elevated exposure ceases. Some data in humans and animals suggest that elimination half-lives for dioxin and related compounds may be dose dependent, with high doses being eliminated more rapidly than lower doses. Nonetheless, the use of an approximately 7-year half-life of elimination presents a reasonable approach for evaluating both back-calculated and average lifetime levels, because for most cohorts the exposure is primarily to TCDD.

The ability to detect effects in epidemiologic study is dependent on a sufficient difference between control and exposed populations. The relatively small difference (<10-100 fold) between exposed and controls in these studies makes exposure characterization in the studies a particularly serious issue. This point also strengthens the importance of measured blood or tissue levels in the epidemiologic analyses, despite the uncertainties associated with calculations extending the distribution of measured values to the entire cohort and assumptions involved in back-calculations.

Characterization of the risk of exposure of humans today remains focused on the levels of exposure that occur in the general population, with particular attention given to special populations (see Part I). For evaluation of multiple endpoints and considering the large differences in half-lives for TCDD across multiple species, it is generally best to use body burden rather than daily intake as the dose metric for comparison unless data to the contrary are presented. Further discussion of this point, which provides the rationale for this science-based policy choice, is presented in Part II, Chapters 1 and 8.

5.1.1. Calculations of Effective Dose (ED)

Comparisons across multiple endpoints, multiple species, and multiple experimental protocols are too complicated to be made on the basis of the full dose-response curve. As discussed above, comparisons of this sort can be made by either choosing a given exposure and

comparing the responses, or choosing a particular response level and comparing the associated exposures. In the analyses contained in Chapter 8 and elsewhere in the reassessment, comparison of responses is made using estimated exposures associated with a given level of excess response or risk. To avoid large extrapolations, this common level of excess risk was chosen such that for most studies, the estimated exposure is in or near the range of the exposures seen in the studies being compared, with extra weight given to the human data. A common metric for comparison is the effective dose or ED, which is the exposure dose resulting in an excess response over background in the studied population. EPA has suggested this approach in calculating benchmark doses (BMD) (Allen et al., 1994) and in its proposed approaches to quantifying cancer risk (U.S. EPA, 1996). Although effective dose evaluation at the 10% response level (ED_{10} or lower bound on ED_{10} [LED_{10}]) is somewhat the norm, given the power of most chronic toxicology studies to detect an effect, this level is actually higher than those typically observed in the exposed groups in studies of TCDD impacts on humans. To illustrate, lung cancer mortality has a background lifetime risk of approximately 4% (smokers and nonsmokers combined), so that even a relative risk of 2.0 (2 times the background lifetime risk) represents approximately a 4% increased lifetime risk. Based upon this observation and recognizing that many of the TCDD-induced endpoints studied in the laboratory include 1% effect levels in the experimental range, Chapter 8 presents effective doses of 1% or ED_{01}. The use of ED values below 10% is consistent with the Agency's guidance on the use of mode of action in assessing risk, as described in the evaluation framework discussed in Section 3.3, in that the observed range for many "key events" extends down to or near the 1% response level. Determining the dose at which key events for dioxin toxicity begin to be seen in a heterogeneous human population provides important information for decisions regarding risk and safety.

5.2. EMPIRICAL MODELING OF INDIVIDUAL DATA SETS

As described in Chapter 8, empirical models have advantages and disadvantages relative to more ambitious mechanism-based models. Empirical models provide a simple mathematical model that adequately describes the pattern of response for a particular data set and can also provide the means for hypothesis testing and interpolation between data points. In addition, they can provide qualitative insights into underlying mechanisms. However, the major disadvantage is their inability to quantitatively link data sets in a mechanistically meaningful manner. Data available for several biochemical and toxicological effects of TCDD, and on the mechanism of action of this chemical, indicate that there is good qualitative concordance between responses in laboratory animals and humans (see Table 1). For example, human data on exposure and cancer response appear to be qualitatively consistent with animal-based risk estimates derived from carcinogenicity bioassays (see Part II, Chapter 8). These and other data presented throughout this

reassessment would suggest that animal models are generally an appropriate basis for estimating human responses. Nevertheless, there are clearly differences in exposures and responses between animals and humans, and recognition of these is essential when using animal data to estimate human risk. The level of confidence in any prediction of human risk depends on the degree to which the prediction is based on an accurate description of these interspecies extrapolation factors. See Chapter 8 for a further discussion of this point.

Almost all data are consistent with the hypothesis that the binding of TCDD to the AhR is the first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic responses observed in both experimental animals and humans (see Part II, Chapter 2). As such, an analysis of dose-response data and models should use, whenever possible, information on the quantitative relationships among ligand (i.e., TCDD) concentration, receptor occupancy, and biological response. However, it is clear that multiple dose-response relationships are possible when considering ligand-receptor mediated events. For example, dose-response relationships for relatively simple responses, such as enzyme induction, may not accurately predict dose-response relationships for complex responses such as developmental effects and cancer. Cell- or tissue-specific factors may determine the quantitative relationship between receptor occupancy and the ultimate response. Indeed, for TCDD there are much experimental data from studies using animal and human tissues to indicate that this is the case. This serves as a note of caution, as empirical data on TCDD are interpreted in the broader context of complex exposures to mixtures of dioxin-like compounds as well as to non-dioxin-like toxicants.

As for other chemical mechanisms where high biological potency is directed through the specific and high-affinity interaction between chemical and critical cellular target, the supposition of a response threshold for receptor-mediated effects is a subject for scientific debate. The basis of this controversy has been recently summarized (Sewall and Lucier, 1995).

Based on classic receptor theory, the occupancy assumption states that the magnitude of biological response is proportional to the occupancy of receptors by drug molecules. The "typical" dose-response curve for such a receptor-mediated response is sigmoidal when plotted on a semilog graph or hyperbolic if plotted on a arithmetic plot. Implicit in this relationship is low-dose linearity (0-10% fractional response) through the origin. Although the law of mass action predicts that a single molecule of ligand can interact with a receptor, thereby inducing a response, it is also stated that there must be some dose that is so low that receptor occupancy is trivial and therefore no perceptible response is obtainable.

Therefore, the same receptor occupancy assumption of the classic receptor theory is interpreted by different parties as support for and against the existence of a threshold. It has been stated that the occupancy assumption cannot be accepted or rejected on experimental or theoretical grounds (Goldstein et al., 1974). To determine the relevance of receptor interaction

for TCDD-mediated responses, one must consider (1) alternatives as well as limitations of the occupancy theory; (2) molecular factors contributing to measured endpoints; (3) limitations of experimental methods; (4) contribution of measured effect to a relevant biological/toxic endpoint; and (5) background exposure.

Throughout this reasssessment, each of these considerations has been explored within the current context of the understanding of the mechanism of a action of TCDD, of the methods for analysis of dose-response for cancer and noncancer endpoints, and of the available data sets of TCDD dose and effect for several rodent species, as well as humans that were occupationally exposed to TCDD at levels exceeding the exposure of the general population.

5.2.1. Cancer

As described in Section 2.2.1.4, TCDD has been classified as a human carcinogen, and is a carcinogen in all species and strains of laboratory animals tested. The epidemiological database for TCDD, described in detail in Part II, Chapter 7a, suggests that exposure may be associated with increases in all cancers combined, in respiratory tumors and, perhaps, in soft-tissue sarcoma. Although there are sufficient data in animal cancer studies to model dose-response for a number of tumor sites, as with many chemicals, it is generally difficult to find human data with sufficient information to model dose-response relationships. For TCDD, there exist three studies of human occupational exposure with enough information to perform a quantitative dose-response analysis. These are the NIOSH study (Fingerhut et al., 1991a), the Hamburg cohort study (Manz et al., 1991), and the BASF cohort study (Zober et al., 1990). In Part II, Chapter 8, simple empirical models were applied to these studies for which exposure-response data for TCDD are available in human populations.

Modeling cancer in humans uses slightly different approaches from those used in modeling animal studies. The modeling approach used in the analysis of the human epidemiology data for all cancers combined and lung cancer involves applying estimated human body burden to cancer response and estimating parameters in a linear risk model for each data set. A linear risk model was used because the number of exposure groups available for analyses was too small to support more complicated models. Because of this, evaluating the shape of the dose-response data for the human studies was not done. Access to the raw data may make it possible to use more complicated mathematical forms that allow for the evaluation of shape. In the one case in which this has been done, the dose-response shape suggested a response that was less than linear (dose raised to a power <1) (Becher et al., 1998). For these studies, there are several assumptions and uncertainties involved in modeling the data, including extrapolation of dosage, both in back-calculation and in elimination kinetics, and the type of extrapolation model employed.

As described in Part II, Chapter 8, the data used in the analyses are from Aylward et al. (1996) for the NIOSH study, Flesch-Janys et al. (1998) for the Hamburg cohort, and Ott and Zober (1996a,b) for the BASF cohort. The limited information available from these studies is in the form of standard mortality ratios (SMRs) and/or risk ratios by exposure subgroups with some estimate of cumulative subgroup exposures. Exposure subgroups were defined either by number of years of exposure to dioxin-yielding processes or by extrapolated TCDD levels. No study sampled TCDD blood serum levels for more than a fraction of its cohort, and these samples were generally taken decades after last known exposure. In each study, serum fat or body fat levels of TCDD were back calculated using a first-order model. The assumed half-life of TCDD used in the model varied from study to study. Aylward et al. used the average TCDD levels of those sampled in an exposure subgroup to represent the entire subgroup. Flesch-Janys et al. and Ott and Zober performed additional calculations, using regression procedures with data on time spent at various occupational tasks, to estimate TCDD levels for all members of their respective cohorts. They then divided the cohorts into exposure groups based on the estimated TCDD levels. The information presented in the literature cited above was used to calculate estimated average TCDD dose levels in Chapter 8.

To provide ED_{01} estimates for comparison in Chapter 8, Poisson regression (Breslow and Day, 1987) was used to fit a linear model to the data described above. Analysis of animal cancer data suggests a mixture of linear and nonlinear responses with linear shape parameters predominating; complex responses to TCDD, both cancer and noncancer, are more often than not nonlinear. Besides the issue of use of a linear model, several other important uncertainties discussed in Chapter 8 are the representativeness and precision of the dose estimates that were used, the choice of half-life and whether it is dose dependent, and potential interactions between TCDD and smoking or other toxicants. Nevertheless, with these qualifications, it is possible to apply simple empirical models to studies in which exposure data for TCDD are available in human populations.

The analysis of these three epidemiological studies of occupationally exposed individuals suggest an effect of TCDD on all cancers, and on lung cancers in the adult human male. The ED_{01}s based upon average excess body burden of TCDD ranged from 6 ng TCDD/kg to 161 ng TCDD/kg in humans. The lower bounds on these doses (based on a modeled 95% C.I.) range from 3.5 ng TCDD/kg to 77 ng TCDD/kg. For the effect of TCDD on lung cancers, the only tumor site increased in both rodents and humans, the human ED_{01}s ranged from 24 ng/kg to 161 ng/kg. The lower bounds on these doses (based on a modeled 95% C.I.) range from 10.5 ng TCDD/kg to 77 ng TCDD/kg. These estimates of ED_{01}s are compared to animal estimates later in this discussion.

Both empirical and mechanistic models were used to examine cancer dose-response in animals. Portier et al. (1984) used a simple multistage model of carcinogenesis with up to two mutation stages affected by exposure to model the five tumor types observed to be increased in the 2-year feed study of Kociba et al. (Sprague-Dawley rats, 1978) and the eight tumor types observed to be increased in the 2-year gavage cancer study conducted by the National Toxicology Program (Osborne-Mendel rats and $B6C3F_1$ mice, 1982a). The findings from this analysis, which examined cancer dose-response within the range of observation are presented in their Table 8.3.2., which is reproduced with slight modifications as Table 5-2. All but one of the estimated ED_{01}s are above the lowest dose used in the experiment (approximately 1 ng TCDD/kg/day in both studies) and are thus interpolations rather than extrapolations. The exception, liver cancer in female rats from the Kociba study, is very near the lowest dose used in this study and is only a small extrapolation (from 1 ng TCDD/kg/day to 0.77 ng TCDD/kg/day). Steady-state body burden calculations were also used to derive doses for comparison across species. Absorption was assumed to be 50% for the Kociba et al. study (feed experiment) and 100% for the NTP study (gavage experiment). Also presented in Table 5-2 are the shapes of the dose-response curves as determined by Portier et al. (1984).

The predominant shape of the dose-response curve in the experimental region is linear; this does not imply that a nonlinear model such as the quadratic or cubic would not fit these data. In fact, it is unlikely that in any one case, a linear model or a quadratic model could be rejected statistically for these cases. These studies had only three experimental dose groups, hence these shape calculations are not based upon sufficient doses to guarantee a consistent estimate; they should be viewed with caution. The ED_{01} steady-state body burdens range from a low value of 14 ng/kg based upon the linear model associated with liver tumors in female rats to as high as 1,190 ng/kg based upon a cubic model associated with thyroid follicular cell adenomas in female rats. Lower bounds on the steady-state body burdens in the animals range from 10 ng TCDD/kg to 224 ng/kg. The corresponding estimates of daily intake level at the ED_{01} obtained from an empirical linear model range from 0.8 to 43 ng TCDD/kg body weight/day depending on the tumor site, species, and sex of the animals investigated. Lower confidence bounds on the estimates of daily intake level at the ED_{01} in the animals range from 0.6 to 14 ng TCDD/kg body weight/day. In addition, using a mechanistic approach to modeling, Portier and Kohn (1996) combined the biochemical response model of Kohn et al. (1993) with a single initiated phenotype two-stage model of carcinogenesis to estimate liver tumor incidence in female Sprague-Dawley rats from the 2-year cancer bioassay of Kociba et al. (1978). By way of comparison, the ED_{01} estimate obtained from this linear mechanistic model was 0.15 ng TCDD/kg body weight/day based on intake, which is equivalent to 2.7 ng TCDD/kg steady-state body burden. No lower bound on this modeled estimate of steady-state body burden was provided.

As discussed in Part II, Chapter 8, different dose metrics can lead to widely diverse conclusions. For example, as described in Chapter 8, the ED_{01} intake for the animal tumor sites presented above ranges from less than 1 to tens of ng/kg/day, and the lowest dose with an increased tumorigenic response (thyroid tumors) in a rat is 1.4 ng/kg/day (NTP, 1982a). The daily intake of TCDD in humans is estimated to be 0.14 to 0.4 pg TCDD/kg/day. This implies that humans are exposed to doses 3,500 to 10,000 times lower than the lowest tumorigenic daily dose in rat thyroid. However, 1.4 ng/kg/d in the rat leads to a steady-state body burden of approximately 25 ng/kg, assuming a half-life of TCDD of 23 days and absorption from feed of 50%[2]. If the body burden of TCDD in humans is approximately 5 ng TCDD/kg lipid or 1.25 ng/kg body weight (assuming about 25% of body weight is lipid), humans are exposed to about 20 times less TCDD than the minimal carcinogenic dose for the rat. If total TEQ is considered the difference is even less, approaching only a factor of 2 difference. The difference between these two estimates is entirely due to the approximately 100-fold difference in the half-life between humans and rats. At least for this comparison, if cancer is a function of average levels in the body, the most appropriate metric for comparison is the average or steady-state body-burden, since the large differences in animal to human half-life are accounted for. Comparisons of human and animal ED_{01}s from Part II, Chapter 8, for cancer response on a body-burden basis show approximately equal potential for the carcinogenic effects of TCDD. In humans, restricting the analysis to log-linear models in Part II, Chapter 8, resulted in cancer ED_{01}s ranging from 6 ng/kg to 161 ng/kg. This was similar to the empirical modeling estimates from the animal studies, which ranged from 14 ng/kg to 1,190 ng/kg (most estimates were in the range from 14 to 500 ng/kg). The lower bounds on the human body-burdens at the ED_{01}s (based on a modeled 95% C.I.) range from 3.5 ng TCDD/kg to 77 ng TCDD/kg. Lower bounds on the steady-state body burdens in the animals range from 10 ng TCDD/kg to 224 ng/kg. The estimate for the single mechanism-based model presented earlier (2.7 ng/kg) was approximately 2 times lower than the lower end of the range of human ED_{01} estimates and less than the lower bound on the LED_{01}. The same value was approximately 5 times lower than the lower end of the range of animal ED_{01} estimates and less than 4 times less than the LED_{01}.

Using human and animal cancer ED_{01}s, their lower bound estimates, and the value of 2.7 ng TCDD/kg from the single mechanism-based model, slope factors and comparable risk estimates for a human background body burden of approximately 5 ng TEQ/kg (20 ng TEQ/kg lipid) can be calculated using the following equations:

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[2] Steady-state body burden (ng/kg) = (daily dose (ng/kg/day) * (half-life)/Ln(2)) (f), where f is the fraction absorbed from the exposure route (unitless) and half-life is the half-life in days.

Slope factor (per pg TEQ/kgBW/day) = risk at ED_{01} / intake (pg TEQ/kgBW/day) associated with human equivalent steady-state body burden at $ED_{01,}$ where:

Risk at ED_{01} = 0.01; and

$$\text{Intake (pgTEQ/kgBW/day)} = \left[\frac{\text{body burden at } ED_{01} \text{ (ng TEQ/kg)} * \text{half-life (days)}}{\frac{\text{Ln}(2)}{\text{half-life}}}\right] * f \quad (5\text{-}1)$$

half-life = 2,593 days in humans and 25 days in rats (see Table 8.1 in Part II, Chapter 8)

f = fraction of dose absorbed; assumed to be 50% for absorption from food (Kociba et al., 1976) and 100% for other routes.

Upper bound on excess risk at human background body burden = (human background body burden (ng/kg))(risk at ED_{01})/lower bound on human equivalent steady-state body burden (ng/kg) at ED01, where: (5-2)

Risk at ED_{01} = 0.01

Use of these approaches reflects methodologies being developed within the context of the revised draft Cancer Risk Assessment Guidelines. Slopes are estimated by a simple proportional method at the "point of departure" (LED_{01}) at the low end of the range of experimental observation. As discussed below, these methods can be compared to previous approaches using the linearized multistage (LMS) procedure to determine if the chosen approach has significantly changed the estimation of slope. The estimates of ED_{01}/LED_{01} represent the human-equivalent body burden for 1% excess cancer risk based on exposure to TCDD and are assumed for purposes of this analysis to be equal for TCDD equivalents (total TEQ). This assumption is based on the toxicity equivalence concept discussed throughout this report and in detail in Part II, Chapter 9. All cancer slope factors can be compared to the Agency's previous slope factor of 1.6×10^{-4} per pgTCDD/kgBW/day (or 1.6×10^{5} per mgTCDD/kgBW/day) (U.S. EPA, 1985).

5.2.1.1. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on Human Data*

Estimates of upper bound slope factors (per pg TCDD/kgBW/day) calculated from the human ED_{01}s presented in Table 8.3.1 range from 5.3×10^{-3}, if the LED_{01} for all cancer deaths in the Hamburg cohort is used, to 2.4×10^{-4} if the ED_{01} for lung cancer deaths in the smaller BASF cohort is used. All of the other slope factors for all cancer deaths or lung cancer deaths in the three cohorts would fall within this range. LED_{01}s for all cancer deaths span approximately an

order of magnitude and would generate slope factors in the range of 5×10^{-3} to 5×10^{-4}. Slightly smaller slope factors are generated when LED_{01}s for lung cancer are used. The largest slope factors based on LED_{01}s come from the Hamburg cohort (5.3×10^{-3} and 1.8×10^{-3} respectively for all cancer deaths and lung cancer deaths.) These estimates compare well with the estimates of risk associated with TCDD exposure in the Hamburg cohort published by Becher et al. (1998). The risk estimates of Becher et al. derived from data on TCDD exposure to male workers with a 10-year latency and taking greater caution over other factors affecting risk including choice of model, latency, job category, dose metric, and concurrent exposures. These estimates range from 1.3×10^{-3} to 5.6×10^{-3} per pg TCDD/kgBW/day. In this analysis all excess cancers are attributed to TCDD exposure, despite significant levels of other dioxin-like compounds in blood measurements of this cohort (see Table 5-1). Although risk estimates using TCDD alone in this cohort might suggest an overestimate of risk, no evidence for this emerged from the analysis and, assuming that TCDD will still dominate total TEQ in this population, differences in slope factor estimates are likely to be less than a factor of 2 and may not be discernable. Taking into account different sources of variation, Becher et al. (1998) suggest a range of 10^{-3} to 10^{-2} for additional lifetime cancer risk for a daily intake of 1 pg TCDD/kg BW/day. By inference, that range could also apply to total TEQ intake. As described in Section 4.4.2, current estimates of intake in the United States are estimated to be approximately 1 pg TEQ/kg BW/day. Using Equation 5-2, the upper bound range of risks estimated from current human body burdens of 5 ng TEQ/kgBW (which equates to a serum level of 20 pg/g lipid [see Table 4.7]) based on all cancer deaths in the three cohorts ranged from 1.4×10^{-2} to 1.3×10^{-3}; based on lung cancer deaths, the upper bound on the estimates of excess risk extended to 6×10^{-4}. The range of these estimates provides further support for the perspective on risk provided by Becher et al. (1998). Uncertainties associated with these estimates from human studies are discussed in Part II, Chapter 8, and in Becher et al. (1998).

5.2.1.2. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on Animal Data*

Upper bound slope factors (per pg TCDD/kgBW/day) for human cancer risk calculated from lower bounds in ED_{01}s ($LEDs_{01}$) for the animal cancers presented in Table 5-2 range from 1.9×10^{-3} to 8.4×10^{-5}. This spans a range from being 12 times greater than the previous upper bound estimate on cancer slope (1.6×10^{-4} [U.S. EPA, 1985]) to 2 times less. The largest slope factor is derived from the same study as the 1985 estimate; that is, the slope factor derived from the female liver cancer in the Kociba et al. (1978) study continues to give the largest slope factor. In attempting these comparisons, two issues became apparent. First, the body burden and the intake at the ED_{01} from Portier et al. (1984) does not result in the same slope factor as U.S. EPA

(1985). Despite the use of the same study results, a slope factor of 1.8×0^{-5} per pg TCDD/kgBW/day results using the LMS approach. This is a factor of approximately 10 lower than the EPA (1985) estimate of the slope. The differences are attributable to the aims of the respective calculations at the time. Portier et al. (1984) calculated "virtually safe doses" assuming that rodent and human doses scaled on a mg/kg basis, and he used the original tumor counts from the study. EPA (1985), on the other hand, used $(BW)^{2/3}$ to arrive at a human equivalent dose and used the pathology results from a reread of the original Kociba study (U.S. EPA, 1980). In addition, tumor counts were adjusted for early mortality in the study. The factor to adjust for $(BW)^{3/4}$-scaling in the rat is 5.8. The correction for early mortality can be accounted for with a factor of 1.6 (this is the ratio of the intake values at the ED01 with and without the early mortality correction). If the Portier et al. slope factor (1.8×10^{-5} per pg TCDD/kgBW/day) is multiplied by these two factors, a slope of 1.7×10^{-4} per pg TCDD/kgBW/day is calculated. This is equivalent to the U.S. EPA (1985) estimate of 1.6×10^{-4} per pg TCDD/kgBW/day. Reconciling these issues is important to ensure appropriate comparisons of slope factor estimates.

More important is the calculation of slope factor estimates using current methods of analysis that recognize the importance of the dose metric and the differences in half-life of dioxins in the bodies of laboratory animals and humans (see Part II, Chapter 8, for detailed discussion). The major difference between the approaches used to calculate risks in the mid-1980s (Portier et al., 1984; U.S. EPA, 1985) and the current approach is the use of body burden as the dose metric for animal-to-human dose equivalence. All things being equal, the use of body burden accounts for the approximately 100-fold difference between half-lives of TCDD in humans and rats (2,593 days versus 25 days [see Part II, Table 8.1]). Use of Equation 5-1 results in an estimated body burden at the LED_{01} of 6.1 ng TEQ/kg to be derived from the EPA (1985) Kociba tumor counts. This compares favorably with the Portier estimate of 10 ng TEQ/kg found in Table 5-2. The difference is entirely accounted for by the early deaths adjustment by EPA (1985). Use of these body burdens at the LED_{01} results in slope factor estimates of 1.9×10^{-3} per pg TCDD/kgBW/day and 3.1×10^{-3} per pg TCDD/kgBW/day for the Chapter 8 and the newly derived body burden, respectively. Again, the difference is due solely to the adjustment for early mortality and EPA believes this provides a better estimate of upper bound lifetime risk than does the unadjusted. EPA's new slope factor (3.1×10^{-3} per pgTCDD/kgBW/day) is 19 times greater than the slope factor from 1985.

A second issue with the modeling of the Kociba data relates to the appropriate tumor counts to use. As mentioned in Section 2, Goodman and Sauer (1992) reported a second re-evaluation of the female rat liver tumors in the Kociba study using the latest pathology criteria for such lesions. Results of this review are discussed in more detail in Part II, Chapter 6. The review confirmed only approximately one-third of the tumors of the previous review (U.S. EPA,

1980). Although this finding did not change the determination of carcinogenic hazard because TCDD induced tumors in multiple sites in this study, it does have an effect on evaluation of dose-response and on estimates of risk. Because neither the original EPA (1985) slope factor estimate nor that of Portier et al. (1984) reflect this reread, it is important to factor these results into the estimate of the ED_{01} and slope factor. Using the LMS procedure used by EPA in 1985 and the tumor counts as reported in Part II, Chapter 6, Table 6.2, the revised slope factor is reduced by approximately 3.6-fold to yield a slope factor of 4.4×10^{-5} per pg TCDD/kgBW/day. However, because the original estimates used a $(BW)^{3/4}$ scaling, this must be adjusted to use body burden and obtain an appropriate result. When dose is adjusted and Equation 5-1 is used, an LED_{01} of 22.2 ng TEQ/kg and a slope factor of 8.3×10^{-4} per pg TCDD/kgBW/day are derived. This represents EPA's most current upper bound estimate of human cancer risk based on animal data. It is 5.2 times larger than the slope factor calculated in U.S. EPA (1985). This number reflects the increase in slope factor based on use of the body burden dose metric (19 times greater) and the use of the Goodman and Sauer (1992) pathology (3.6 times less).

5.2.1.3. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on a Mechanistic Model*

As discussed above, Portier and Kohn (1996) combined the biochemical response model of Kohn et al. (1993) with a single initiated-phenotype two-stage model of carcinogenesis to estimate liver tumor incidence in female Sprague-Dawley rats from the Kociba et al. (1978) bioassay. The model is described in more detail in Part II, Chapter 8. This model adequately fit the tumor data, although it overestimated the the observed tumor response at the lowest dose in the Kociba study. The shape of the dose-response curve was approximately linear and the estimated ED_{01} value for this model was 1.3 ng/kg/day. The corresponding body burden giving a 1% increased effect was 2.7 ng/kg. The model authors believe that the use of CYP1A2 as a dose metric for the first mutation rate is consistent with its role as the major TCDD-inducible estradiol hydrolase in liver and with its hypothesized role in the production of estrogen metabolites leading to increased oxidative DNA damage and increased mutation (Yager and Liehr, 1996; Hayes et al., 1996; Dannan et al., 1986; Roy et al., 1992). Although no lower bound estimate of the ED_{01} is calculated, a maximum likelihood estimate of the slope factor can be calculated. It is 7.1×10^{-3} per pg TCDD/kgBW/day. This estimate represents an example of the type of modeling, based on key events in a mode of action for carcinogenesis, which is consistent with future directions in dose-response modeling described in EPA's revised proposed cancer risk assessment guidelines (U.S. EPA, 1999). Although a number of uncertainties remain regarding structure and parameters of the model, the slope estimate is consistent with those derived from humans and animals. More details on this model can be found in Part II, Chapter 8.

5.2.2. Noncancer Endpoints

At this point, sufficient data are not available to model noncancer endpoints in humans. Many studies are available to estimate ED_{01} values for noncancer endpoints in animals. However, there are a number of difficulties and uncertainties that should be considered when comparing the same or different endpoints across species. Some of these include differences in sensitivity of endpoints, times of exposure, exposure routes, species and strains, use of multiple or single doses, and variability between studies even for the same response. The estimated ED_{01}s may be influenced by experimental design, suggesting that caution should be used in comparing values from different designs. In addition, caution should be used when comparing studies that extrapolate ED_{01}s outside the experimental range. Furthermore, it may be difficult to compare values across endpoints. For example, the human health risk for a 1% change of body weight may not be equivalent to a 1% change in enzyme activity. Finally, background exposures are not often considered in these calculations simply because they were not known. Nevertheless, given these considerations, several general trends were observed and discussed in Part II, Chapter 8. The lowest ED_{01}s tended to be for biochemical effects, followed by hepatic responses, immune responses, and responses in tissue weight. An analysis of shape parameters implies that many dose-response curves are consistent with linearity over the range of doses tested. This analysis does not imply that the curves would be linear outside this range of doses, but it does inform the choices for extrapolation. This is particularly true when body burdens or exposures at the lower end of the observed range are close to body burdens or exposures of interest for humans, which is the case with dioxin-like chemicals.

Overall, shape parameter data suggest that biochemical responses to TCDD are more likely to be linear within the experimental dose range, while the more complex responses are more likely to assume a nonlinear shape. However, a large number (> 40%) of the more complex responses have shape parameters that are more consistent with linearity than nonlinearity.

The tissue weight changes seen for animals (using only data sets with good or moderate empirical fits to the model) yielded a median ED_{01} at average body burdens of 510 ng/kg in the multidose studies (range; 11 to 28000 ng/kg) and a median ED_{01} of 160 ng/kg (range 0.0001 to 9,700 ng/kg) in the single dose studies. Toxicity endpoints from the single dose studies resulted in a median value at average body burdens of 4,300 ng/kg (range 1.3 to 1,000,000 ng/kg). For tissue weight changes, 43% of the dose-response curves exhibited linear response. In contrast, the toxicity endpoints from the single-dose studies exhibited predominantly nonlinear responses (80%). All multidose studies demonstrated a greater degree of linear response (41%) than did single-dose studies (37%), especially for tissue weight changes and toxicity endpoints (50% linear for multidose versus 34% for single dose). In general, it is not possible to dissociate the

differences between cancer and noncancer dose-response as being due to differences in endpoint response or simply to differences in the length of dosing and exposure. Also, a greater percentage of the noncancer ED_{01}s were extrapolations below the lower range of the data (42%) than was the case for the cancer endpoints (8% in animals and no extrapolations in humans).

5.3. MODE-OF-ACTION BASED DOSE-RESPONSE MODELING

As described in Chapter 8, mechanism-based modeling can be a powerful tool for understanding and combining information on complex biological systems. Use of a truly mechanism-based approach can, in theory, enable reliable and scientifically sound extrapolations to lower doses and between species. However, any scientific uncertainty about the mechanisms that the models describe is inevitably reflected in uncertainty about the predictions of the models. The assumptions and uncertainties involved in the mechanistic modeling described in Chapter 8 are discussed at length in that chapter and in cited publications.

The development and continued refinement of PBPK models of the tissue dosimetry of dioxin have provided important information concerning the relationships between administered does and dose to tissue compartments (section 8.2). Aspects of these models have been validated in the observable response range for multiple tissue compartments, species, and class of chemical. These models will continue to provide important new information for future revisions of this health assessment document. Such information will likely include improved estimates of tissue dose for liver and other organs where toxicity has been observed, improved estimates of tissue dose(s) in humans, and improved estimates of tissue dose for dioxin related compounds.

As a part of this reassessment, the development of biologically based dose-response (pharmacodynamic) models for dioxin and related compounds has lead to considerable and valuable insights regarding both mechanisms of dioxin action and dose-response relationships for dioxin effects. These efforts, described in some detail in Chapter 8, have provided additional perspectives on traditional methods such as the linearized multistage procedure for estimating cancer potency or the uncertainty factor approach for estimating levels below which noncancer effects are unlikely to occur. These methods have also provided a biologically based rationale for what had been primarily statistical approaches. The development of models like those in Chapter 8 allows for an iterative process of data development, hypotheses testing and model development.

5.4. SUMMARY DOSE-RESPONSE CHARACTERIZATION

All humans tested contain detectable body burdens of TCDD and other dioxin-like compounds that are likely to act through the same mode of action. It is possible that any additional exposure above current background body burdens will be additive to ongoing responses. The magnitude of the additional response will be a function of the toxicity equivalence

of the incremental exposure. This observation, the relatively small margin of exposure for "key events," and the high percentage of observed linear responses suggest that a proportional model should be used when extrapolating beyond the range of the experimental data. Short of extrapolating to estimate risk in the face of uncertainties described above, a simple margin-of-exposure approach may be useful to decision-makers when discussing risk management goals. However, this decision would have to be based upon a policy choice because this analysis does not strongly support either choice.

Because human data for cancer dose-response analysis were available and because of a strong desire to stay within the range of responses estimated by these data, the risk chosen for determining a point of departure was the 1% excess risk. Doses and exposures associated with this risk (the ED_{01}s) were estimated from the available data using both mechanistic and empirical models. Comparisons were made on the basis of body burdens to account for differences in half-life across the numerous species studied.

In humans, restricting the analysis to log-linear models resulted in cancer ED_{01}s ranging from 6 ng/kg to 161 ng/kg. This was similar to the estimates, from empirical modeling, from the animal studies which ranged from 14 ng/kg to 1,190 ng/kg (most estimates were in the range from 14 to 500 ng/kg), and 2.7 ng/kg for the single mechanism-based model. Lower bounds on these ED_{01} estimates were used to calculate upper bound slope factors and risk estimates for average background body burdens. These estimates are presented above. Upper bound slope factors allow the calculation of the probability of cancer risk for the highly vulnerable in the population (estimated to be the top 5% or greater). Even though there may be individuals in the population who might experience a higher cancer risk on the basis of genetic factors or other determinants of cancer risk not accounted for in epidemiologic data or animal studies, the vast majority of the population is expected to have less risk per unit of exposure and some may have zero risk. Based on these slope factor estimates (per pg TEQ/kgBW/day), average current background body burdens (5 ng/kgBW) that result from average intakes of approximately 3 pgTEQ/kgBW/day are in the range of 10^{-3} to 10^{-2}. A very small percentage of the population (< 1%) may experience risks that are 2-3 times higher than this if they are among both the most vulnerable and the most highly exposed (among the top 5%) based on dietary intake of dioxin and related compounds. This range of upper bound risk for the general population has increased an order of magnitude from the risk described at background exposure levels based on EPA's draft of this reassessment (10^{-4}-10^{-3}) (U.S. EPA, 1994).

Estimates for noncancer endpoints showed much greater variability, ranging over 10 orders of magnitude. In general, the noncancer endpoints displayed lower ED_{01}s for short-term exposures versus longer term exposures, and for simple biochemical endpoints versus more complex endpoints such as tissue weight changes or toxicity. In addition, the noncancer

endpoints generally displayed higher estimated ED_{01}s than the cancer endpoints, with most estimates ranging from 100 ng/kg to 100,000 ng/kg. The mechanism-based models for noncancer endpoints gave a lower range of ED_{01}s (0.17 to 105 ng/kg). Although most of these estimates were based upon a single model the estimate from the hepatic zonal induction model gave an ED_{01} for CYP1A2 induction of 51 ng/kg and hence was within the same range.

These estimates, although highly variable, suggest that any choice of body burden, as a point of departure, above 100 ng/kg would likely yield >1% excess risk for some endpoint in humans. Also, choosing of a point of departure below 1 ng/kg would likely be an extrapolation below the range of these data and would likely represent a risk of <1%. Any choice in the middle range of 1 ng/kg to 100 ng/kg would be supported by the analyses, although the data provide the greatest support in the range of 10 ng/kg to 50 ng/kg.